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Responses of soil enzymes to long-term CO_2 enrichment in forest ecosystems of Changbai Mountains

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Abstract: A study was conducted to determine the responses of soil enzymes (invertase, polyphenol oxidase, catalase, and dehydrogenase) to long-term CO_2 enrichment at the Research Station of Changbai Mountain Forest Ecosystems, Chinese Academy of Sciences (42°24′N, 128°28′E; 738 m in elevation) in the northeast China during 1999–2006. Three treatments of the CO_2 enrichment, designed as 500 μmol·mol^{-1} CO_2 open-top chamber (OTC), ambient control chamber and unchambered field (approx. 370 μmol·mol^{-1}CO_2), were conducted with *Pinus koraiensis* and *Pinus sylvestriformis* tree species. Soil sampling was made and analyzed separately in spring, summer and autumn in 2006 after the soil enzymes were exposed to elevated CO_2 concentration (500 μmol·mol^{-1}) for eight growing seasons. Results showed that, at elevated CO_2 concentration (500 μmol·mol^{-1}), the activities of invertase (except for the summer samples of *P. koraiensis*) presented a remarkable decline in all growing seasons, while the activities of dehydrogenase had an increase but only part of the results was remarkable; the activities of polyphenol oxidase in *P. sylvestriformis* rhizosphere showed a remarkable decrease; the catalase activities increased in spring, while in turn were decline in other seasons. This study also revealed that the soil enzyme activities are significantly correlated with the tree species under the CO_2 enhancement.

Keyword: CO_2 concentration; CO_2 enrichment; Soil enzymes; Invertase; Dehydrogenase; Catalase; Polyphenol oxidase

Introduction

The atmospheric CO_2 concentration has been increasing since the middle of last century due to fossil fuel burning and land-use change. Currently, the CO_2 concentration is rising at the rate of 1.5 μmol·mol^{-1} per year on average (IPCC 2001). Direct effects of elevated CO_2 on soil organisms are unlikely because CO_2 concentration in soils is already 10–50 times higher than that in the atmosphere (Lanborg *et al*. 1983). Three plant-mediated mechanisms increasing atmospheric CO_2 concentration might influence soil microbial communities. Firstly, the elevated CO_2 stimulates plant photosynthesis, and consequently increases net primary productivity. At least part of the extra C fixed is allocated blow-ground. This can result in an increase in root biomass, root-shoot ratio, fine root biomass and fine root turnover (Rogers *et al*. 1994). Secondly, chemistry of green leaves is altered. The ratio of carbon to nitrogen increases in green leaf tissues partly due to starch accumulation. As soil microorganisms are often constrained by available C (Paul and Clark 1996), it is likely that they respond to these changes by increasing biomass and/or activity. However, naturally senesced litter often does not show these changes in C/N (Hirschel *et al*. 1997; Norby *et al*. 2001). Also, the concentration of phenolic compounds such as lignin and tannins sometimes increases, reducing the decomposability of the plant material. Thirdly, the elevated CO_2 reduces stomatal conductance of plants, which results in higher water use efficiency. At a plant community level, this often decreases stand transpiration and maintains higher soil water content (Körner 2000). Increased soil moisture is beneficial to soil microbes and their activity (Killham 1994). Plant responses to elevated CO_2 have been widely studied, and results generally varied for different species under different nutritional conditions. Microbial responses to elevated CO_2 in complex natural ecosystems are less well understood (Kampichler *et al*. 1998). Zak *et al*. (2000) reported that the response of soil microorganisms to elevated CO_2 is highly variable, no matter whether activity, biomass or effects on the N-cycle were studied. This variability cannot be explained by plant life-forms. Studies showed that the changes of soil microbial parameters at elevated CO_2 often dealt with soil-plant systems that are characterized by high underground carbon-input from plants in combination with low carbon content of the soil (Zak *et al*. 2000). In contrast, there are few studies about the soil microbial response to elevated CO_2 in China, and the articles mostly originate from short-term experiments. Extrapolations of these results to mature ecosystems and to longer time scales are scant (Hu *et al*. 1999).

Soil microorganisms hold a key position in terrestrial ecosystems as they mineralize organic matter. Therefore, any effect of elevated CO_2 on soil microorganisms could in turn feed back the response of plant communities to CO_2 enrichments, thus seques-

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trating extra carbon. Though microbial decomposition and mineralisation are mediated by soil enzymes, relatively few studies involved in the measurements of enzyme activities (e.g. KÖrner and Arnone 1992; Kandeler *et al*. 1998; Kang *et al*. 2001). The objective of the present study is to assess the effect of elevated CO_2 on soil enzyme activities after long-term exposure to CO_2 enrichment in situ in Changbai Mountain Ecosystem and the difference in enzyme activities between different tree species.

Materials and methods

Study site and experimental design

The study site is situated at the Research Station of Changbai Mountain Forest Ecosystems, Chinese Academy of Sciences (42°24′N, 128°28′E; 738 m in elevation) in the northeast China. The climate of this area is characterized by temperate zone continental climate, with cold and lengthy winter and warm and rainy summer. The mean annual precipitation is around 700 mm, mean annual temperature is 3.5 °C, and the frost-free period is about 100–200 days.

The CO_2 enrichment experiment was conducted in 1999 by three treatments, 500 μmol·mol^{-1} CO_2 open-top chamber (OTC), ambient control chamber and unchambered field (approx. 370 μmol·mol^{-1}CO_2). The treatment 500 μmol·mol^{-1}CO_2 had been consecutively operated 24 h every day during the whole growing season from May to October since 1999. The target CO_2 concentration was monitored by automatic controlled system.

The tree species chosen for the experiment were eight years old *Pinus koraiensis* and. The average height was 60 cm for *P. koraiensis* and 170 cm for *P. sylvestriformis*. They were daily irrigated except rainy day.

Soil sampling and storage

Soil was separately sampled in the middle of May (spring), middle of June (summer), and the middle of September 2006 (autumn), when the filed experiment was terminated. From each plot, three subsamples, each containing approximately 100 g soil, were taken from the top 10 cm of the horizon. The samples were kept at room temperature after sieving 2 mm and air-dry,.

Measurements of soil enzymes activities

Invertase activity was measured by incubating 5 g dry soil with 15 ml of 8% sucrose for 24 h at 37°C. When the production reacted to 3,5-dinitrosalicylic acid, the colorimetric determination was conducted.

The activity of polyphenol oxidase was determined by incubating 5 g dry soil with 10 mL of a 0.02 mM catechol for 2 min at 30℃. The action was stopped with 3 mL of a 10% phosphoric acid, and the production was measured by iodimetry with starch indicator.

The activity of catalase was measured by incubating 2 g dry soil with 5 ml of a 0.3% H_2O_2 for 20 min with continuously shaking. The action was stopped with 5 ml of a 3 N H_2SO_4, and the production was titrated by 0.1 N K_2MO_4.

The measurement of dehydrogenase was made by incubating 6 g dry soil with 1 ml of a 3% 2, 3, 5-triphenyltetrazdium chloride (TTC) for 24 h at 37 ºC, then washed the producing soil with carbinol, last colorimetric determination.

Three repeats were made for each test and each variety.

Statistical analyses

Duncans multiple range test was used for mean comparison if the results of the *F*-test were significant at the 0.05 level.

Results

Fig. 1 and Fig. 2 show the response of soil enzyme activities of a forestland to elevated atmospheric CO_2 during eight growing seasons. The change rules of soil enzyme activities are different to various enzymes and tree species.

The response of soil enzyme activities to elevated atmospheric CO_2 in *Pinus koraiensis* rhizosphere

To *Pinus koraiensis*, in spring, the activities of invertase and polyphenol oxidase presented a strong response to the CO_2 enhancement with a significant decrease (P<0.05). The activities of polyphenol oxidase and dehydrogenase increased with the elevated CO_2 concentration. In summer, the activities of all enzymes, except catalase, measured at all sampling dates were higher under elevated CO_2 than those in the ambient control chamber. In autumn, the activities of invertase and catalase under elevated CO_2 were 14.5% and 3% lower than those in the ambient control chamber, respectively; the activities of polyphenol oxidase and dehydrogenase increased insignificantly at elevated CO_2 (Fig. 1).

The response of soil enzyme activities to elevated atmospheric CO_2 in *Pinus sylvestriformis* rhizosphere

To *Pinus sylvestriformis*, in spring, the activities of invertase and polyphenol oxidase under elevated CO_2 were 23% and 22% lower than those in the ambient control chamber, respectively, while the activity of catalase at elevated CO_2 increased by 16% compared to that in the ambient control chamber. In summer, the activities of invertase and polyphenol oxidase under elevated CO_2 were 21% and 17% lower than those in the ambient control chamber, respectively. In autumn, the activities all enzymes except dehydrogenase measured at all sampling dates were lower under elevated CO_2 than those in the ambient control chamber (Fig. 2).

Discussion

The processes of biology and biochemistry are the very important base of terrestrial ecosystems in soil. Elevated concentration of atmospheric CO_2 have distinct effect on soil physical and chemical properties, soil biology communities and plants, thus it directly or indirectly affect soil enzymes (Bazzaz 1990).

The soil invertase is the biocatalyst, which can reflect the relationship of soil carbon and soil respiration The result that elevated CO_2 can enhance enzyme activities was also reported by Ross *et al*. (1995) in a short-term chamber experiment with grassland turves exposed to elevated CO_2 for a total of 220 days (700μmol·mol^{-1}CO_2). This was explained by a greater input of plant-derived invertase and greater production of invertase in response to increased C input. In a similar experiment conducted by Ross *et al*. (1996), which lasted 422 days, only minor and insignificant differences in invertase activity were found at different CO_2 concentrations (350, 525, and 700μmol·mol^{-1} CO_2).

To free air carbon dioxide enrichment (FACE), previous studies showed that the invertase activities increased in wheat and rice. However, our experiments suggested that the invertise activities decreased distinctly. This was mainly caused by the differences in time of CO_2 treatment and tree species.

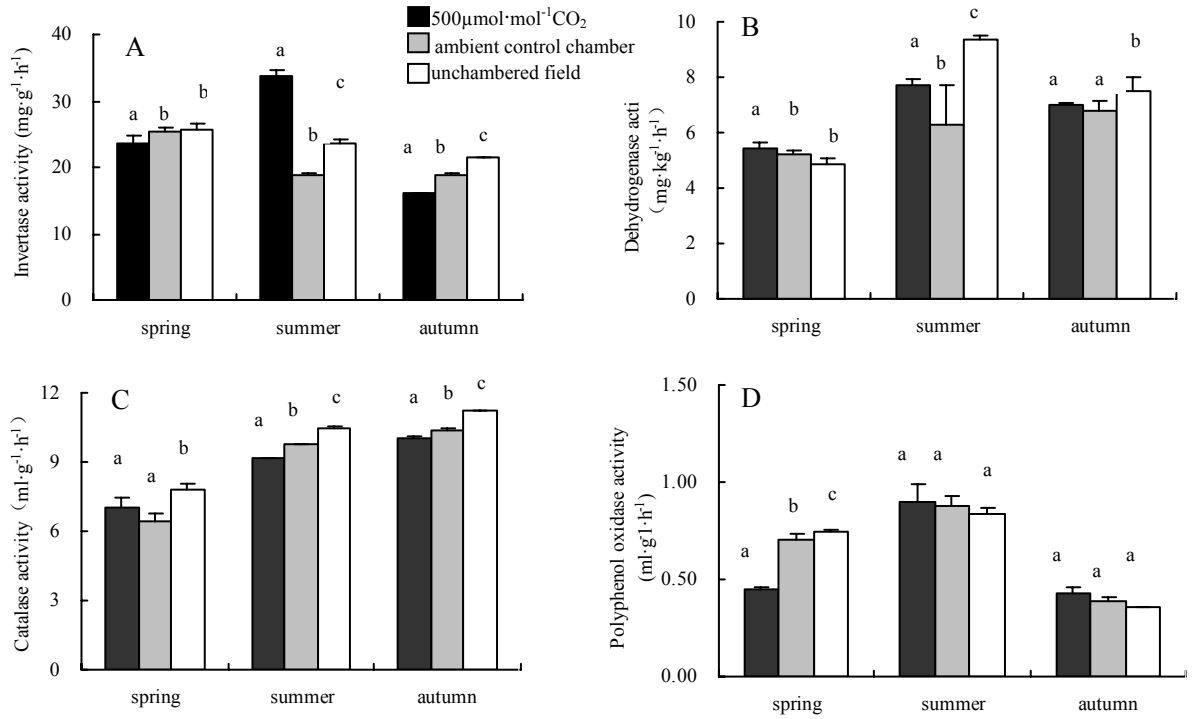

Fig.1 the response of soil enzyme activities of invertase (A), dehydrogenase (B), catalase (C) and polyphenol oxidase (D) to elevated atmospheric CO_2 in *Pinus koraiensis* rhizosphere.

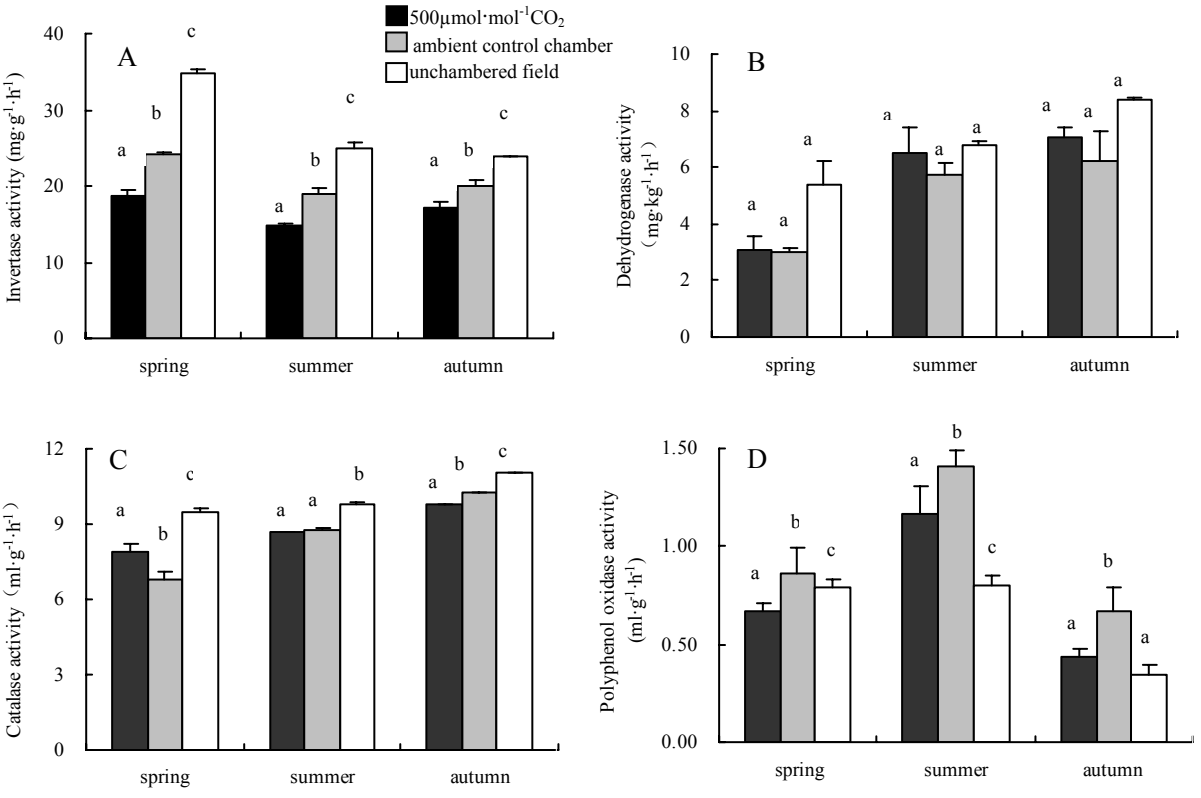

Fig.1 the response of soil enzyme activities of invertase (A), dehydrogenase (B), catalase (C) and polyphenol oxidase (D) to elevated atmospheric CO_2 in *Pinus sylvestriformis* rhizosphere.

The soil dehydrogenase is considered as the index for appraising the soil microbiology activities. Because dehydrogenase only lives in the center of alive cells, Dhillion *et al.* (1996) studied the soil microbiol activity in a Mediterranean model ecosystem. This result showed that, exposed to CO_2 concentrations of 700μmol·mol^{-1} CO_2 for several months, dehydrogenase activities significantly increased by 13%. The result agreed with our observation on soil dehydrogenase activities. The increase in dehydrogenase activities is possiblely correlated with the increase of soil microbioly activities and function diversities in response to increase of the CO_2 concentration.

The soil polyphenol oxidase and catalase were concerned with soil organic matter. Under the elevated CO_2 the decrease in activities of polyphenol oxidase and catalase forecasts that the elevated CO_2 can result in a decline in soil redox and the recomposing ability of humus.

The effect of elevated CO_2 concentration on soil enzyme activities is different between *Pinus koraiensis* and *Pinus sylvestriformis*. It indicated that the soil enzyme activities are significantly correlated with the plant species. Under the elevated CO_2, the net photosynthetic rate, biomass growth rate and root biomass increased, and the change of root secretion can influence the structure of C and N source. Therefore the main reason led to the change of the soil enzyme activities was the microorganism as well as the root.

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